US EPA ARCHIVE DOCUMENT

CATALOG DOCUMENTATION
EMAP-ESTUARIES PROVINCE LEVEL DATABASE
CAROLINIAN PROVINCE 1994-1997
SEDIMENT TOXICITY DATA

TABLE OF CONTENTS

1. DATA SET IDENTIFICATION

1.1 Title of Catalog Document

EMAP-Estuaries Province Level Database
Carolinian Province
Sediment Toxicity Data

1.2 Authors of the Catalog entry

Timothy R. Snoots,
Dr. Jeffrey L. Hyland

1.3 Catalog Revision Date

November 23, 1999

1.4 Data Set Name

CP_TOX.DAT

US EPA ARCHIVE DOCUMENT

1.5 Task Group

Estuaries

1.6 Data set identification codes

7

1.7 Version

001

1.8 Requested Acknowledgment

If you plan to publish these data in any way, EPA requires a standard statement for work it has supported:

"Although the data described in this article have been funded wholly or in part by the U. S. Environmental Protection Agency through its EMAP-Estuaries Program, it has not been subjected to Agency review, and therefore does not necessarily reflect the views of the Agency and no official endorsement should be inferred."

2. INVESTIGATOR INFORMATION

2.1 Principal Investigator

J. Hyland (NOAA/NOS/NCCOS/CCMA) - Carolinian Province Manager
A. Ringwood (SCDNR) - Lead P.I. for SC/GA region team
C. Hackney (UNC-W) - Lead P.I. for NC region team
G. McRae, G. Nelson, J. McKenna, J. Landsberg (FLDEP) - Lead P.I.s for FL region team (depending on year)

2.2 Investigation Participant - Sample Collection

Field Sample Collection

T. Alphin, S. Bowen, C. Byrum, D. Dye, A. Gospodarek, J. Grace, J. Grimley, C. Hackney, C. Powell, C. Preziosi, H. Riley, S. Roberts, M. Smith, K. Stokesbury, D. Tremain, T. Wheeler (UNC-W); S. Ross (NCNERR); M. Armstrong-Taylor, J. Jones, M. Levinson, P. Powers, A. Ringwood, T. Snoots, G. Steele (SCDNR); L. Balthis, T. Herrlinger, C. Keppler, M. Wert (UC); D. Adams, K. Amendola, D. Cook, C. Harnden, B. Heagey, J. Mckenna, G. Nelson, C. Nowicki, R. Paperno, B. Rosenblatt, M. Wessel (FLDEP); J. Hyland, S. Kokkinakis (NOAA/NOS/ORCA)

Field Training and Coordination

S. Kokkinakis (NOAA/NOS/ORCA); J. Macauley (EPA-GED); T. Heitmuller (USGS-GB); D. Keith (EPA-AED)

US EPA ARCHIVE DOCUMENT

2.3 Sampling Processing - Principal Investigator

Program Management and Coordination

J. Hyland, A. Robertson (NOAA/NOS/NCCOS/CCMA); K. Summers (EPA); F. Holland, A. Ringwood (SCDNR); C. Hackney, T. Wheeler (UNC-W); S. Ross (NCNERR); J. Landsberg, J. McKenna, G. McRae, G. Nelson, R. Paperno (FLDEP)

Toxicity Testing

M. DeLorenzo (CU); J. Grimley (UNC-W); J. Jones, C. Keppler, P. Maier, A. Ringwood, R. Van Dolah (SCDNR); P. Ross (Citadel); C. Mueller, J. Scott, G. Thursby (SAIC)

QA/QC

T. Heitmuller (USGS-GB), S. Kokkinakis (NOAA/NOS/ORCA)

Data Management and Statistical Support

T. Snoots, F. Holland, R. VanDolah (SCDNR); L. Balthis, T. Herrlinger (UC); J. Rosen, L. Zimmerman (TPMC); S. Rathbun (UGA); M. Adams, L. Harwell (JCWS); V. Engle (EPA-GED); Z. Malaeb (USGS-GB); S. Hale (EPA-AED); K. Summers (EPA); T. Wilson (CU)

3. DATA SET ABSTRACT

3.1 Abstract of the Data set

The CP_TOX.DAT data set contains results of up to four different toxicity assays (10-day Ampelisca abdita mortality assay, 10-day Ampelisca verrilli mortality assay, 7-day Mercenaria mercenaria growth assay, and Microtox solid-phase assay) performed on sediment samples from each station sampled in the EMAP Carolinian Province from 1994-1997.

The following reports are products of these and other data collected during the 1994-1997 Sampling period in the Carolinian Province. These reports may contain additional information and summary statistics that are not contained in this data set catalog or its respective data sets. We therefore recommend referring to them when using these data.

Hyland, J.L., T.J. Herrlinger, T.R. Snoots, A.H. Ringwood, R.F. Van Dolah, C.T. Hackney, G.A. Nelson, J.S. Rosen, and S.A. Kokkinakis. 1996. Environmental quality of estuaries of the Carolinian Province: 1994. Annual statistical summary for the 1994 EMAP-Estuaries Demonstration Project in the Carolinian Province. NOAA Technical Memorandum NOS ORCA 97. NOAA/NOS, Office of Ocean Resources Conservation and Assessment, Silver Spring, MD. 102 p.

US EPA ARCHIVE DOCUMENT

US EPA ARCHIVE DOCUMENT

Hyland, J.L., L. Balthis, C.T. Hackney, G. McRae, A.H. Ringwood, T.R. Snoots, R.F. Van Dolah, and T.L. Wade. 1998. Environmental quality of estuaries of the Carolinian Province: 1995. Annual statistical summary for the 1995 EMAP-Estuaries Demonstration Project in the Carolinian Province. NOAA Technical Memorandum NOS ORCA 123 NOAA/NOS, Office of Ocean Resources Conservation and Assessment, Silver Spring, MD. 143 p.

See also: Grimley and Hackney (1996), Ringwood et al. (1997b), Ringwood and Keppler (In Press), Ringwood et al.(1998), Ringwood et al. (1996), Ringwoodet al. (1995), Ringwood et al. (1997a), Science Applications International Corp. (1998a), Science Applications International Corp. (1998b).

3.2 Keywords for the Data Set

Sediment toxicity, amphipod, Ampelisca abdita, Ampelisca verrilli, Microtox, Mercenaria mercenaria, EMAP Carolinian Province

4. OBJECTIVES AND INTRODUCTION

4.1 Program Objective

EMAP has three primary objectives:

1. To estimate the current status, extent, changes, and trends in indicators of the Nation's ecological resources on a regional basis;

2. To monitor indicators of pollutant exposure and habitat condition, and to seek correlative relationships between human-induced stresses and ecological condition that identify possible causes of adverse effects; and

3. To provide periodic statistical summaries and interpretive reports on ecological status and trends to the EPA Administrator and to the public.

4.2 Data Set Objective

The CP_TOX.DAT data set contains results of up to four different toxicity assays (10-day Ampelisca abdita mortality assay, 10-day Ampelisca verrilli mortality assay, 7-day Mercenaria mercenaria growth assay, and Microtox solid-phase assay) performed on sediment samples from each station sampled in the EMAP Carolinian Province from 1994-1997.

Where applicable, both assay end results (i.e., % survival, % growth, or EC50), and statistical test results (P-value for test of control vs. sample results) are reported. This allows a duel criteria to be used in the determination of a sample's toxicity (i.e., test results should be both statistically and biologically significant to declare the overall toxicity result

significant). These duel criteria have been applied for each test and results are reported in a coded fashion (1 = significant toxicity, 0 = not toxic).

4.3 Data Set Background Information

The standard 10-day sediment bioassay with the marine amphipod Ampelisca abdita (ASTM 1993) has been used to assess sediment toxicity in other EMAP provinces. This assay was also used in the Carolinian Province in 1994, 1995 and 1997 to provide a basis for comparisons among EMAP provinces and between years within the Carolinian Province. However, because Ampelisca abdita proved to be relatively insensitive to sediment contaminants in prior surveys conducted in both the Carolinian and Louisianian Provinces (Hyland et al. 1996, Macauley et al. 1994), an additional amphipod assay with the congeneric species Ampelisca verrilli was included in the 1995 effort. Preliminary testing with A. verrilli and a subset of the 1994 sediment samples indicated that this species was more sensitive to sediment contamination than A. abdita (Ringwood et al. 1995). Furthermore, A. verrilli is a more common member of the infaunal benthos of southeastern estuaries.

A third bioassay used to measure potential sediment toxicity at all base sites and selected supplemental sites was the Microtox solid-phase test with the photoluminescent bacterium Vibrio fischeri (formerly Photobacterium phosphoreum). This assay provides a sublethal measure of toxicity based on attenuation of light production by the bacterial cells due to exposure to the sediment sample (Bulich 1979, Ross et al. 1991, Microbics 1992a and b). Microtox has not been used in other EMAP-E provinces, but its recent application in other coastal assessment programs suggested that it might be a useful tool to consider for the Carolinian Province. Small sample sizes (a 100-mL subsample of the composited surface sediment from each station) and a short processing time (20-min exposures) provide clear logistical advantages. Results of the Carolinian Province 1993 pilot study (Ringwood et al. 1996) and 1994 monitoring demonstration (Hyland et al. 1996) also suggested that this test is more powerful in its ability to discriminate between degraded and reference sites than the amphipod toxicity test.

A fourth sediment bioassay used in the 1995-1997 surveys was a 7-d sublethal test of the effects of sediment exposure on growth of juvenile Mercenaria mercenaria (referred to hereafter as "seed clams"). The seed-clam bioassay was developed during the Carolinian Pilot Study (Ringwood et al. 1996, Ringwood and Kepler In Press). Field-validation testing on a subset of the 1994 sediment samples indicated that this bioassay was a more sensitive indicator of sediment contamination than the A. abdita bioassay (Ringwood et al. 1995). There are other practical advantages. For example, newly metamorphosed clams exhibit very rapid growth, thus effects on growth can be detected within a short time frame. Second, because seed clams can be obtained from cultured populations (available ~ 3 months after fertilization),

US EPA ARCHIVE DOCUMENT

US EPA ARCHIVE DOCUMENT

experiments can be conducted with animals of similar size, age, and pre-exposure histories. Third, a relatively small sample volume (500 mL) is required, thus minimizing sampling time and storage needs. Lastly, Mercenaria feed at the sediment-water interface, where maximum contaminant exposure would be expected. Thus the bioassay is representative of a realistic exposure scenario.

4.4 Summary of Data Set Parameters

See 4.2 (Data Set Objective)

4.5 Year-Specific Information about Data

Toxicity testing in the Carolinian Province from 1994-1997 was performed by the following laboratories:

	Test Type			
Year	Ampelisca abdita	Ampelisca verrilli	Mercenaria mercenaria	Microtox
1994	SCDNR,SAIC	-	-	SCDNR
1995	SAIC	SCDNR	SCDNR	SCDNR
1996	-	-	SCDNR	UNC-W
1997	SCDNR,SAIC	-	SCDNR	SCDNR,SAIC

Hyphen (-) indicates test was not performed in that year.

5. DATA ACQUISITION AND PROCESSING METHODS

5.1 Data Acquisition

5.1.1 Sampling Objective

See section 4.3 (Data Set Background Information)

5.1.2 Sample Collection Method Summary

A 1/25 m2, Kynar-coated stainless steel, Young Grab sampler was used to collect sediments. This grab sampled an area of 440 cm2 and a maximum depth of penetration in the sediment of 10 cm. Stainless steel utensils were used to remove the top 2-3 cm of sediment from a grab. The sediment was removed to a stainless steel bowl and placed in a cooler of ice to remain cold, but unfrozen. The grab sampler was rinsed and re-deployed. This procedure was repeated until the volume of sediment required for all contaminant, toxicity, and sediment characteristics analyses had been collected. The sediment was mixed by hand until thoroughly homogenized, and aliquots were placed into pre-cleaned containers (Polyethylene for A. abdita and A. verrilli, and Polypropylene for Microtox and M. mercenaria). In addition, Microtox samples were wrapped in foil immediately to exclude light. All toxicity samples were immediately stored on ice following collection in the field, and then refrigerated at 4 C once back in the lab.

5.1.3 Beginning Sampling Dates

30 June 1994
05 July 1995
09 July 1996
07 July 1997

5.1.4 Ending Sampling Dates

31 August 1994
14 September 1995
19 September 1996
25 August 1997

5.1.5 Platform

Samples were collected from various gasoline or diesel powered boats equipped with at least the following equipment: "A" frame boom or davit, winch, LORAN-C or GPS for location, and a depth finder.

5.1.6 Sampling Equipment

A 1/25 m2, Kynar-coated stainless steel, Young Grab sampler. This grab sampled an area of 440 cm2 and a maximum depth of penetration in the sediment of 10 cm.

5.1.7 Manufacturer of Sampling Equipment

Ted Young
Falmouth, MA

5.1.8 Key Variables

5.1.9 Sample Collection Method Calibration

The sampling gear does not require any calibration. It required inspection for deformities incurred due to mishandling or impact on rocky substrates.

5.1.10 Sample Collection Quality Control

Field technicians were trained to follow Standard Operating Procedures to insure the collection of representative, uncontaminated and high quality samples. QA/QC measures were taken in the field to avoid or reduce contamination and insure the collection of representative samples. These included: use of stainless steel instruments, thorough cleaning of the sampler between grabs, use of pre-cleaned containers for sediment storage and ensuring that engines were shut down when a sample was exposed to the air. A successful grab had relatively level, intact sediment over the entire area of the grab and a sediment depth of 7-10 centimeters. Unacceptable grabs included those: containing no sediments, which were partially filled or had shelly substrates or grossly slumped surfaces. Grabs completely

US EPA ARCHIVE DOCUMENT

filled to the top, where the sediment was oozing out of the hinged top, were also unacceptable.

See: Kokkinakis et al. (1994a)

5.1.11 Sample Collection Method References

See: Hyland et al. (1996),
Hyland et al. (1998),
Kokkinakis et al. (1994b)

5.1.12 Sample Collection Method Deviations

None

5.2 Data Preparation and Sample Processing

5.2.1 Sample Processing Objective

Determine toxicity of sediment samples using up to four toxicity assays (10-day Ampelisca abdita mortality assay, 10-day Ampelisca verrilli mortality assay, 7-day Mercenaria mercenaria growth assay, and Microtox solid-phase assay).

5.2.2 Sample Processing Methods Summary

5.2.2.1 Field Summary

NA

5.2.2.2 Laboratory Summary

A. abdita and A. verrilli Testing

Procedures followed the general guidelines provided in ASTM Protocol E1367-92 (ASTM 1993) and the EMAP-E Laboratory Methods Manual (U.S. EPA 1994a, 1994b).This is an acute toxicity test which measures the effect of sediment exposure on amphipod survival under static conditions. Approximately 3-3.5 L of surface sediments (composite of upper 2 cm from multiple grabs) were collected for each type of assay from each station and stored in 3.7-L polyethylene jars at 4 C in the dark until testing. Tests were conducted with subsamples of the same sediment on which analyses of contaminants and other sediment characteristics were performed. Wherever possible, sediment samples were tested within 30 days of collection as recommended in the EMAP-E protocol.

Amphipods were collected from unpolluted tidal flats. Prior to testing, the animals were acclimated at 20 C for 2-9 days in the case of A. abdita, or for 2-4 days in the case of A. verrilli. During the acclimation period, the amphipods were fed the diatom Phaeodactylum tricornutum. Wherever possible, juvenile amphipods of approximately the same size (usually 3-5 mm in length for A. abdita, and 3-10 mm in length for A. verrilli)

US EPA ARCHIVE DOCUMENT

US EPA ARCHIVE DOCUMENT

were used to initiate the tests.

The general health of each batch of amphipods was evaluated by a reference toxicity test (i.e., "positive control"). These tests were run in a dilution series with seawater (no sediment phase) and the reference toxicant sodium dodecyl sulfate (SDS). Tests for both species were run under static conditions in dark and followed the basic methods described by ASTM (1993). The exposure period was 96 h for A. abdita and 24 h for A. verrilli. The shorter exposure period was used for A. verrilli to match previous reference toxicant tests conducted with this species by MRRI. LC50 values were computed for each batch of test animals for comparison against background toxicity data on these same species and reference toxicant. Animals were not used in definitive tests with field samples unless acceptable reference toxicant results were obtained. A test was considered acceptable if the LC50 value was within +/- 2 SD of the mean LC50 based on the preceding 20 (A. abdita) to 22 (A. verrilli) reference toxicant tests.

Treatments for the definitive tests with field samples consisted of a single concentration of each sediment sample (100% sediment) and a negative control [i.e., reference sediment]. A negative control was run with each batch of field samples. The tests were conducted under static conditions at a temperature of 20 +/- 1 C and salinity range of 26-33ppt for A. abdita and 26-35ppt for A. verrilli. Twenty amphipods were randomly distributed to each of five replicates per each treatment including the control. Amphipods were not fed during the tests.

The negative controls provided a basis of comparison for determining statistical differences in survival in the field sediments. In addition, control survival provided a measure of the acceptability of final test results. Test results were considered valid if mean control survival (among the 5 replicates) was >= 85% and survival in any single control chamber was >= 80%.

One-liter glass containers with covers were used as test chambers. Each chamber was filled with 200 mL of sediment and 600-800 mL of filtered seawater. The sediment was press-sieved through a 2.0-mm screen to remove ambient fauna prior to placing it in a chamber. All containers were illuminated constantly throughout the 10-day test to inhibit amphipod emergence from the sediment, thus maximizing exposure to the test sediment. Air was supplied using oil-free aerators and glass pipettes inserted into the test chambers. Water tables with recirculating chiller pumps were used to maintain constant temperatures (20 +/- 1 C). Daily recordings were made of temperature and the number of dead vs. living animals. On days two and eight, two of the five

replicate chambers for each treatment were selected randomly and measured for salinity, dissolved oxygen, pH, and total ammonia in the overlying water.

At the conclusion of a test, the sediment from each chamber was sieved through a 0.5-mm screen to remove amphipods. The number of animals dead, alive, or missing was recorded. Sediments with missing A. abdita were preserved in formalin containing Rose Bengal stain and re-examined under a dissecting microscope to ensure that no living specimens had been missed. Animals still unaccounted for were considered to have died and decomposed in the sediment. Because of their larger size, A. verrilli were much easier to locate with the unaided eye. Thus, if any of these animals were missing after initial examination of the sieved sediment, then they were assumed to have died and decomposed.

Differences between survival of Ampelisca abdita in field versus control samples were evaluated by an unpaired heteroscedastic t-test run on untransformed percentage data, under the assumptions of normality and unequal variances. For A. verrilli, differences between field samples and controls were evaluated by either: (i) an unpaired homoscedastic t-test in cases of normal data with equal variances, or (ii) a Mann-Whitney U-test in cases of non-normal data or unequal variances. The A. verrilli comparisons also were performed on untransformed percentage data. For both bioassays, field samples were considered to be significantly toxic if mean survival in comparison to the corresponding negative control was < 80% and statistically different at alpha = 0.05.

A variety of quality control procedures were incorporated to assure acceptability of amphipod test results and comparability of the data with other studies. As described above, these provisions included the use of standard ASTM and EMAP protocols, positive controls run with a reference toxicant, negative "performance" controls run with reference sediment, and routine monitoring of water quality variables to identify any departures from optimum tolerance ranges. In addition, during the first year of the program, an inter-laboratory comparison of results using the A. abdita assay was performed by the two participating testing facilities (SAIC and SCDNR/MRRI). Samples from two of the base sites collected in 1994 were tested by each facility. Results were highly comparable: mean survival in field samples relative to controls was 96% for both samples by one lab, and 98 to 100% by the other lab.

US EPA ARCHIVE DOCUMENT

US EPA ARCHIVE DOCUMENT

Microtox Testing

Tests were conducted in duplicate following the "large-sample-size" protocol of Microbics Corporation (1992b). Wherever possible, sediment samples were tested within the recommended 10-d holding period. A 7-g aliquot of each sediment sample was used to make a dilution series ranging from 0.01 to 10% sediment in a 2% saline diluent. A reagent solution containing the bacteria was then added to each sediment suspension. After a 20-min incubation period, a column filter was used to separate the liquid phase and bacterial cells from the sediment. Post-exposure light output in each of the filtrates was measured on a Microtox Model 500 Analyzer. A log-linear regression model was used to determine an EC50 - the sediment concentration that reduced light production by 50% relative to a control (nontoxic reagent blank). EC50 values were corrected for percent water content and reported as dry-weight concentrations.

Assays were run with the reference toxicant phenol with each new batch of bacteria. These tests provided measures of the general quality of the bacterial populations, as well as the ability of the laboratory to produce results consistent with the expected phenol toxicity range (i.e., Microtox EC50 values typically between 13-26 mg/L). Use of the standard Microtox equipment and protocol helped to assure data comparability with results of other Microtox studies.

Mercenaria mercenaria Testing

Seed clams (~ 1 mm in length) were obtained from Atlantic Clam Farms, Folly Beach, S.C. Replicate subsets were dried and weighed to provide initial weight estimates. On the day before initiation of a test, sediment samples were sieved through a 500-micrometer screen (to remove ambient fauna) and distributed to the test chambers. Approximately 50 mL of sieved sediment were added to each of four replicate 250-mL beakers for each sediment sample. A negative control (same Folly River sediments used as controls in the Ampelisca verrilli assays) was run with each batch of field samples. Filtered seawater (1-micrometer filter), adjusted to 25 ppt with deionized water, was added to each beaker to bring the total volume up to 200 mL. The sediment suspension was allowed to settle overnight and clams (30-50 per replicate) were added the next day (which initiated the test).

Tests were conducted for 7 d. All tests were conducted at room temperature (23-25 C) under gentle aeration. Animals were fed three times throughout the test with a phytoplankton mixture consisting of equal volumes of Isochrysis galbana and Chaetocerus gracilis.

At the end of the 7-d exposure period, clams were sieved from the sediments, placed in clean seawater, and allowed to depurate for ~ 1 h. Clams were re-captured on a sieve and rinsed briefly with distilled water to remove excess salt. Dead clams were removed and not included in subsequent growth estimates (mortality rates generally were < 10%). The remaining live clams were dried overnight (60-70 C), counted, and weighed on a micro-balance. The pre- and post-exposure measurements were then used to determine growth rates, expressed as ug/clam/d. Effects of sediment exposure on growth rates were evaluated using either a t-test or Mann-Whitney U-test (when assumptions of the parametric test were violated). Samples were considered to be significantly toxic if mean growth rate in comparison to the control was < 80% and statistically different at alpha = 0.05.

Each new batch of seed clams was evaluated for suitability and relative sensitivity with a reference toxicant test ("positive control"). These tests were run under static conditions, at room temperature, in a dilution series with 25ppt seawater (no sediment phase) and the reference toxicant cadmium. Treatments within each test consisted of a seawater control and four cadmium concentrations (25, 50, 100, 200 ug/L as CdCl2). Each treatment was represented by 3-4 replicates. The effective Cd concentration that reduced growth by 50% (EC50) relative to the seawater control was estimated by regression analysis.

5.2.3 Sample Processing Method Calibration

See Section 5.2.2.2 (Sample Processing Methods Summary/ Laboratory Summary)

5.2.4 Sample Processing Quality Control

See Section 5.2.2.2 (Sample Processing Methods Summary/ Laboratory Summary)

5.2.5 Sample Processing Method Reference

See Section 5.2.2.2 (Sample Processing Methods Summary/ Laboratory Summary)

5.2.6 Sample Processing Method Deviations

None

US EPA ARCHIVE DOCUMENT

6. DATA ANALYSIS AND MANIPULATIONS

6.1 Name of New or Modified Value

P_VALUE	P-value for statistical test
RESULT	Test result (relative to control)
SIG	Toxicity Test Result (1=Hit)

6.2 Data Manipulation Description

P_VALUE

For A. abdita and A. verrilli, P_VALUE is the probability value associated with a statistical test of mean survival of Amphipods in field versus control samples. The test performed was either: (1) an unpaired homoscedastic t-test in cases of normal data with equal variances, or (2) a Mann-Whitney U-test in cases of non-normal data or unequal variances. The A. verrilli comparisons were performed on untransformed percentage data.

For M. mercenaria, P_VALUE is the probability value associated with a statistical test of mean growth of M. mercenaria in field versus control samples. Effects of sediment exposure on growth rates were evaluated using either a t-test or Mann-Whitney U-test (when assumptions of the parametric test were violated).

RESULT

For A. abdita and A. verrilli, RESULT is mean survival in field sediment as a percent of mean survival in control sediment (e.g., RESULT = field/control * 100)

For Microtox, values reported in RESULT are EC50 values (sediment concentration that reduced light production by 50% relative to a control). These EC50 values have been corrected for percent water content and are reported as percent (dry-weight) sediment concentrations.

For M. mercenaria, RESULT is mean clam growth in field sediment as a percent of mean growth in control sediment (e.g., RESULT = field/control * 100).

SIG

The variable SIG reports a final, coded toxicity result based on a duel criteria of statistical significance (P_VALUE) and biological significance (RESULT). If both the P_VALUE and RESULT for an assay meet the criteria for significance, then the response is considered to be significantly toxic and SIG = 1. If either P_VALUE or RESULT for an assay do not meet the appropriate criteria for significance, then the response is considered not toxic and SIG = 0. Where available data are not adequate to evaluate using this duel criteria approach (e.g., P_VALUE or RESULT data are missing), SIG = "." (missing). The criteria used to evaluate toxicity responses in each of the

US EPA ARCHIVE DOCUMENT

four assays are discussed below.

For both A. abdita and A.verrilli assays, field samples were considered to be significantly toxic if mean survival of field samples as a percent of the mean survival for the corresponding negative control (RESULT) was < 80% and statistically different at alpha = 0.05 (P_VALUE).

For Microtox assays, evaluation criteria were established for two separate silt-clay classes because of the strong inverse relationship between Microtox EC50 values and percent silt-clay content. Sediments with >= 20% silt-clays were classified as being toxic if EC50 values were <= 0.2%; sediments with < 20% silt-clays were classified as being toxic if EC50 values were <= 0.5% (sensu Ringwood et al. 1995). Lower EC50 values in muddier sediments are believed to be caused by physical adsorption of the bacteria to the sediment particles. Ringwood et al. (1995, 1997) demonstrated this effect by conducting Microtox assays in artificial sediment mixtures of pure sand and kaolin clay and evaluating the EC50 values as a function of the finer-particle content.

For M. mercenaria assays, samples were considered to be significantly toxic if mean growth rate in field samples as a percent of mean growth rate in the controls (RESULT) was < 80% and statistically different at alpha = 0.05 (P_VALUE).

6.3 Data Manipulation Examples

NA

7. DATA DESCRIPTION

7.1 Description of Parameters

Variable	Type	Format	Label
DATE	Num	YYMMDD6.	Sample collection date (YYMMDD)
P_VALUE	Num	6.4	P-value for statistical test
QC_CODE	Char	30.	QC Code
RESULT	Num	8.3	Test result (relative to control)
SIG	Num	1.	Toxicity Test Result (1=Hit)
STA_NAME	Char	7.	Carolinian Province Office Station Name
TEST	Char	22.	Test Species
UNIT	Char	10.	Test result unit code

Note the conventions used in the Format column above:

For character (Char) variables, the number given is the maximum width (number of characters) for that variable.

For numeric (Num) variables, the format is given in W.D format, where W = maximum width (number of characters) for the number (including all digits and the decimal point), and D = number of digits to the right of the decimal point.

US EPA ARCHIVE DOCUMENT

7.1.6 Precision to which values are reported

Variable RESULT is reported to 0.001 units, however, values are only valid to: 0.1 units for A. abdita and A. verrilli results, 0.01 units for M. mercenaria results, and 0.001 units for Microtox results. Variable P_VALUE is reported to 0.0001 units, however, values are only valid to: 0.001 units for A. abdita and A. verrilli results, and 0.0001 units for M. mercenaria results.

7.1.7 Minimum Value in Data Set

Variable	Minimum
P_VALUE	0.0000
RESULT	-148.690
SIG	0

7.1.8 Maximum Value in Data Set

Variable	Maximum
P_VALUE	1.0000
RESULT	219.420
SIG	1

7.2 Data Record Example

7.2.1 Column Names for Example Records

```
STA_NAME;DATE;TEST;RESULT;UNIT;P_VALUE;SIG;QC_CODE
```

7.2.2 Example Data Records

```
CP95121;950720;Ampelisca abdita;104.200;Survival_%;0.0790;0;AST-E
CP95121;950720;Ampelisca verrilli;103.000;Survival_%;0.2000;0;
CP95121;950720;Mercenaria mercenaria;135.320;Growth_%;.;0;CST-E, CST-F
CP95121;950720;Microtox (V. fischeri);0.550;EC50_%;.;0;
CP95122;950720;Ampelisca abdita;100.000;Survival_%;0.5000;0;AST-E
CP95122;950720;Ampelisca verrilli;97.000;Survival_%;0.3700;0;
CP95122;950720;Mercenaria mercenaria;78.710;Growth_%;0.0286;1;CST-F
CP95122;950720;Microtox (V. fischeri);0.640;EC50_%;.;0;
CP95123;950726;Ampelisca abdita;92.500;Survival_%;0.0080;0;
CP95123;950726;Ampelisca verrilli;104.000;Survival_%;0.3700;0;
CP95123;950726;Mercenaria mercenaria;52.540;Growth_%;0.0024;1;CST-F
CP95123;950726;Microtox (V. fischeri);9.520;EC50_%;.;0;
```

8. GEOGRAPHIC AND SPATIAL INFORMATION

8.1 Minimum Longitude

-81 Degrees, 43.83 Minutes West Longitude

US EPA ARCHIVE DOCUMENT

8.2 Maximum Longitude

-75 Degrees, 33.82 Minutes West Longitude

8.3 Minimum Latitude

27 Degrees, 12.07 Minutes North Latitude

8.4 Maximum Latitude

36 Degrees, 43.43 Minutes North Latitude

8.5 Name of area or region

Coastal distribution of sampling is along the southeastern US from Cape Henry, VA, through St. Lucie Inlet, FL. States represented: Virginia, North Carolina, South Carolina, Georgia, and Florida.

9. QUALITY CONTROL/QUALITY ASSURANCE

9.1 Measurement Quality Objectives

See Section 5.2.2.2 (Sample Processing Methods Summary/ Laboratory Summary)

9.2 Quality Assurance/Control Methods

See Section 5.2.2.2 (Sample Processing Methods Summary/ Laboratory Summary)

9.3 Quality Assessment Results

Unless flagged with one of the following QC codes, all data reported in the CP_TOX.DAT data set met the QA/QC guidelines given above and are acceptable without further qualification.

Where necessary, the following QC codes are reported under the variable QC_CODE and should be considered when interpreting test results.

QC Code	Description
AST-C	Fewer than 5 replicates tested
AST-E	Sample held for longer than 30 days
AST-M	More than 20 animals inoculated into replicate
CST-A	Fewer than 4 replicates were tested
CST-B	Sediment too coarse to sieve through 0.5 mm mesh therefore making it difficult to recover clams.
CST-C	Sediment held longer than 30 days prior to testing
CST-D	Folly River control sediment not used. Note that this occurred only once. EXPTNUM 950928, sediment from Breach Inlet used.
CST-E	Statistical analysis not run because the mean growth rate was >100% of the mean control growth rate

US EPA ARCHIVE DOCUMENT

CST-G	Very high to complete mortality of clams in sample (i.e., sample is toxic)
CST-H	Fewer than 3 replicates were tested (cadmium exposures only)
MST-A	Samples were processed within 14 days of sampling
MST-E	Sample held for > 10 days prior to testing
MST-F	Unable to calculate an EC50 value for this sample due to an insignificant decrease in luminescence or an increase in luminescence (i.e., little or no toxic effects)
MST-X	Calculated EC50 result was greater than the highest test concentration of 10%. Because the accuracy of an EC50 value above 10% is unknown, EC50 values greater that 10% have been reported as 10.000%.
MST-Y	Hit/Miss result could not be determined due to missing silt-clay data

---

10. DATA ACCESS

10.1 Data Access Procedures

Data can be downloaded from the WWW site.

10.2 Data Access Restrictions

Data can only be accessed from the WWW site.

10.3 Data Access Contact Persons

For programmatic/policy matters, contact:
Dr. Jeffrey L. Hyland
NOAA/NOS National Centers for Coastal Ocean Science
Center for Coastal Monitoring and Assessment - Charleston Lab
217 Fort Johnson Rd. (P.O. Box 12559)
Charleston, SC 29422-2559
(843)762-5415 (Tel.)
(843)762-5110 (FAX)
jeff.hyland@noaa.gov (e-mail)

For data-related questions, contact:
Dr. W. Leonard Balthis
NOAA/NOS National Centers for Coastal Ocean Science
Center for Coastal Monitoring and Assessment - Charleston Lab
217 Fort Johnson Rd. (P.O. Box 12559)
Charleston, SC 29422-2559
(843)762-5652 (Tel.)
(843)762-5110 (FAX)
len.balthis@noaa.gov (e-mail)

US EPA ARCHIVE DOCUMENT

Data Librarian, EMAP-Estuaries
Melissa M. Hughes
OAO Corporation
U.S. EPA NHEERL-AED
27 Tarzwell Drive
Narragansett, RI 02882-1197
(401) 782-3184 (Tel.)
(401) 782-3030 (FAX)
hughes.melissa@epa.gov (e-mail)

10.4 Data file Format

Delimited ASCII Text

10.5 Information Concerning Anonymous FTP

Not accessible

10.6 Information Concerning WWW

Data can be downloaded from the WWW.

10.7 EMAP CD-ROM Containing the Data file

Data not available on CD-ROM.

11. REFERENCES

American Society for Testing and Materials (ASTM). 1993. Guide for conducting 10-day static sediment toxicity tests with marine and estuarine amphipods. ASTM Standard Method E-1367-92, ASTM, Philadelphia, PA. 24p.

Bulich, A.A. 1979. Use of luminescent bacteria for determining toxicity in aquatic environments. In: L. L. Marking and R. A. Kimerle (eds.), Aquatic Toxicology, pp. 98-106. ASTM STP 667. American Society for Testing and Materials, Philadelphia, PA

Grimley, J. and C.T. Hackney. 1996 Year end summary. EMAP Carolinian Province: North Carolina Estuaries Component. Final year-three report under NOAA Cooperative Agreement No. NA47OA0148. University of North Carolina at Wilmington, Center for Marine Research, Wilmington, NC.

Hyland, J.L., T.J. Herrlinger, T.R. Snoots, A.H. Ringwood, R.F. Van Dolah, C.T. Hackney, G.A. Nelson, J.S. Rosen, and S.A. Kokkinakis. 1996. Environmental quality of estuaries of the Carolinian Province: 1994. Annual statistical summary for the 1994 EMAP-Estuaries Demonstration Project in the Carolinian Province. NOAA Technical Memorandum NOS ORCA 97. NOAA/NOS, Office of Ocean Resources Conservation and Assessment, Silver Spring, MD. 102 p.

US EPA ARCHIVE DOCUMENT

US EPA ARCHIVE DOCUMENT

Hyland, J.L., L. Balthis, C.T. Hackney, G. McRae, A.H. Ringwood, T.R. Snoots, R.F. Van Dolah, and T.L. Wade. 1998. Environmental quality of estuaries of the Carolinian Province: 1995. Annual statistical summary for the 1995 EMAP-Estuaries Demonstration Project in the Carolinian Province. NOAA Technical Memorandum NOS ORCA 123 NOAA/NOS, Office of Ocean Resources Conservation and Assessment, Silver Spring, MD.

Kokkinakis, S.A., C. Mageau, and A. Robertson. 1994a. Carolinian Demonstration Project - 1994 Quality Assurance Project Plan. Joint National Status and Trends/Environmental Monitoring and Assessment Program. NOAA/NOS/ORCA, Silver Spring, MD.

Kokkinakis, S.A., J.L. Hyland, and A. Robertson. 1994b. Carolinian Demonstration Project - 1994 Field Operations Manual. Joint National Status and Trends/Environmental Monitoring and Assessment Program. NOAA/NOS/ORCA, Silver Spring, MD.

Macauley, J.M., J.K. Summers, P.T. Heitmuller, V.D. Engle, G.T. Brooks, M. Babikow, and A.M. Adams. 1994. Annual Statistical Summary: EMAP - Estuaries Louisiana Province - 1992. U.S. EPA Office of Research and Development, Environmental Research Laboratory, Gulf Breeze, FL. EPA/620/R-94/002.

Microbics Corporation. 1992a. Microtox Manual (5 volume set). Carlsbad, CA.

Microbics Corporation. 1992b. Microtox Update Manual, 128 p. Carlsbad, CA.

Ringwood, A.H., M.E. DeLorenzo, P.E. Ross, and A.F. Holland. 1997b. Interpretation of Microtox« solid-phase toxicity tests: the effects of sediment composition. Environ. Toxicol. Chem. 16(6):1135-1140.

Ringwood, A.H. and C. Keppler. In Press. Seed clam growth: A sediment bioassay developed in the EMAP Carolinian Province, Environ. Monitor. & Assess, Submitted May 1997.

Ringwood, A.H., C. Keppler, R.F. Van Dolah, P. Maier, J. Jones, M.E. DeLorenzo. 1998. EMAP Activities in the Carolinian Province by Marine Resources Research Institute in 1997: Results and Summaries. Final Annual Report under NOAA Cooperative Agreement No. NA770A0214. South Carolina Department of Natural Resources, Marine Resources Research Institute, Charleston, SC.

Ringwood, A.H., A.F. Holland, R. Kneib, and P. Ross. 1996. EMAP/NS&T pilot studies in the Carolinian Province: Indicator testing and evaluation in southeastern estuaries. Final Report under Grant NA90AA-D-SG790 through S.C. Sea Grant College Program. S.C. Dept. of Natural Resources, Marine Resources Research Institute, Charleston, S.C. NOAA Technical Memorandum NOS ORCA 102. 113 p.

Ringwood, A.H., R. Van Dolah, A.F. Holland, and M.G. Delorenzo. 1995. Year one demonstration project studies conducted in the Carolinian Province by Marine Resources Research Institute: Results and summaries. Final Annual Report under NOAA Cooperative Agreement No. NA47OA0177. South Carolina Department of Natural Resources, Marine Resources Research Institute, Charleston, S.C

Ringwood, A.H., R. Van Dolah, A.F. Holland, M.G. Delorenzo, C. Keppler, P. Maier, J. Jones, and M. Armstrong-Taylor. 1997a. Year two demonstration project studies conducted in the Carolinian Province by Marine Resources Research Institute: Results and summaries. Final Annual Report under NOAA Cooperative Agreement No. NA47OA0177. South Carolina Department of Natural Resources, Marine Resources Research Institute, Charleston, SC.

Science Applications International Corp. 1998a. Sediment toxicity testing (MAIA 1997), Amphipod test results, final report (21 Jan 1998). Science Applications International Corporation, Narragansett, RI.

Science Applications International Corp. 1998b. Sediment toxicity testing (MAIA 1997), Microtox test results, final report (10 Feb 1998). Science Applications International Corporation, Narragansett, RI.

Ross, P.E., L.C. Burnett, C. Kermode, and M. Timme. 1991. Miniaturizing a toxicity test battery for screening contaminated sediments. Can. Tec. Rep. Fish. Aquat. Sci., 1774: 331-335.

Strobel, C.J., S.J. Benyi, D.J. Keith, H.W. Buffum, and E.A. Petrocelli. 1994. Statistical summary: EMAP - Estuaries Virginian Province - 1992. U.S. EPA Office of Research and Development, Environmental Research Laboratory, Narragansett, RI. EPA/62O/R-94/O19.

Summers, J.K., J.M. Macauley, P.T. Heitmuller, V.D. Engle, A.M. Adams, and G.T. Brooks. 1993. Annual statistical summary: EMAP - Estuaries Louisianian Province - 1991. U.S. EPA Office of Research and Development, Environmental Research Laboratory, Gulf Breeze, FL. EPA/600/R-93/001.

U.S. EPA. 1994a. Environmental Monitoring and Assessment Program (EMAP): Laboratory methods manual - estuaries, Volume 1: Biological and physical analyses. Office of Research and Development, Environmental Monitoring and Systems Laboratory, Cincinnati, OH. EPA/62O/R-94/xxx.

U.S. EPA. 1994b. Methods for assessing the toxicity of sediment-associated contaminants with estuarine and marine amphipods. EPA 600/R-94/025. U.S. EPA Office of Research and Development, Narragansett, R.I.

US EPA ARCHIVE DOCUMENT

U.S. EPA. 1995. Environmental Monitoring and Assessment Program (EMAP): Laboratory Methods Manual-Estuaries, Volume 1: Biological and Physical Analyses. U.S. Environmental Protection Agency, Office of Research and Development, Narragansett, RI. EPA/620/R-95/008.

12. TABLE OF ACRONYMS

<=	Less than or equal to
>=	Greater than or equal to
C	Degrees Celsius
cm2	Square centimeters
CMBAD	Coastal Monitoring and Bioeffects Assessment Division
CU	Clemson University
EMAP	Environmental Monitoring and Assessment Program
EPA	U.S. Environmental Protection Agency
EPA-AED	EPA-Atlantic Ecology Division
EPA-GED	EPA-Gulf Ecology Division
EPA-RTP	EPA-Research Triangle Park, NC
FLDEP	Florida Dept. of Environmental Protection
FMRI	Florida Marine Research Institute
FTP	File Transfer Protocol
GIS	Geographical Information System
JCWS	Johnson Controls Word Services
km2	Square kilometers
m2	Square meters
mg/L	Milligrams per liter
mS/cm	MilliSiemens per centimeter (equiv. to milliohms/cm)
MRRI	Marine Resources Research Institute
NCNERR	North Carolina National Estuarine Research Reserve
NCSU	North Carolina State University, NC
NA	Not Applicable
ng/g	Nanograms per gram
NOAA	National Oceanic and Atmospheric Administration
NOS	National Ocean Service
ORCA	Office of Ocean Resources Conservation and Assessment
QA/QC	Quality Assurance/Quality Control
ppb	Parts per billion (equiv. to ng/g)
ppm	Parts per million (equiv. to ug/g)
ppt	Parts per thousand
SAIC	Science Applications International Corporation
SCDNR	South Carolina Dept. of Natural Resources
TOC	Total Organic Carbon
TAMU/GERG	Texas A&M University, Geochemical and Environmental Research Group
TPMC	Technology Planning and Management Corporation
ug/g	Micrograms per gram
um	Micrometers
UC	University of Charleston, SC
UGA	University of Georgia, GA
UNC-W	University of North Carolina - Wilmington, NC
USGS-GB	US Geological Survey - Gulf Breeze, FL
wt.	Weight
WWW	World Wide Web -Internet

US EPA ARCHIVE DOCUMENT

13. PERSONNEL INFORMATION

Dr. Courtney T. Hackney
University of North Carolina - Wilmington,
Wilmington, NC

Melissa M. Hughes
Data Librarian, EMAP-Estuaries
OAO Corporation
U.S. EPA NHEERL-AED
27 Tarzwell Drive
Narragansett, RI 02882-1197
(401) 782-3184 (Tel.)
(401) 782-3030 (FAX)
hughes.melissa@epa.gov (e-mail)

For programmatic/policy matters, contact:
Dr. Jeffrey L. Hyland
NOAA/NOS National Centers for Coastal Ocean Science
Center for Coastal Monitoring and Assessment - Charleston Lab
219 Fort Johnson Rd.
Charleston, SC 29412-9110
(843)762-8652 (Tel.)
(843)762-8700 (FAX)
jeff.hyland@noaa.gov (e-mail)

For data-related questions, contact:
Dr. W. Leonard Balthis
NOAA/NOS National Centers for Coastal Ocean Science
Center for Coastal Monitoring and Assessment - Charleston Lab
219 Fort Johnson Rd.
Charleston, SC 29412-9110
(843)762-8654 (Tel.)
(843)762-8700 (FAX)
len.balthis@noaa.gov (e-mail)

Jan Landsberg
Florida Dept. of Environmental Protection
Florida Marine Research Institute
St. Petersburg, FL

James McKenna
Florida Dept. of Environmental Protection
Florida Marine Research Institute
St. Petersburg, FL

Dr. Gil McRae
Florida Dept. of Environmental Protection
Florida Marine Research Institute
St. Petersburg, FL

Gary A. Nelson
Florida Dept. of Environmental Protection
Florida Marine Research Institute
St. Petersburg, FL

US EPA ARCHIVE DOCUMENT

Dr. Amy H. Ringwood
South Carolina Dept. of Natural Resources,
Marine Resources Research Institute
Charleston, SC

US EPA ARCHIVE DOCUMENT